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Award Number: W81XWH-11-1-0387

TITLE: Viral Oncolytic Therapeutics for Neoplastic Meningitis

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REPORT DATE: July 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

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1. REPORT DATE 01-Jul-2012	2. REPORT TYPE Annual	3. DATES COVERED 1 July 2011 – 30 June 2012		
4. TITLE AND SUBTITLE Viral Oncolytic Therapeutics for Neoplastic Meningitis		5a. CONTRACT NUMBER		
		5b. GRANT NUMBER W81XWH-11-1-0387		
		5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) M. Papisov E-Mail: Papisov@helix.mgh.harvard.edu		5d. PROJECT NUMBER		
		5e. TASK NUMBER		
		5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Massachusetts General Hospital Boston, Massachusetts 02114		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)		
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT The goal of this collaborative exploratory project is to develop novel, safe and efficient therapy for neoplastic meningitis (meningeal metastasis of breast cancer). The proposed therapy will be based on direct (intrathecal) administration of oncolytic viruses into the cerebrospinal fluid (CSF). During the first research year, physiology of the intrathecal delivery of particles was studied in rats. The data was compared with the results obtained in non-human primates in our parallel studies. The key findings are the following. (1) The key factor defining the patterns of the initial particle distribution between the cerebral and spinal CSF is the volume of the bolus. (2) The key physiological factors defining further transport of the particles in the leptomeningeal space (and further into perivascular spaces) are tissue pulsation and particle surface structure. The data of the functional investigation of the diameter of meningeal pores draining CSF to the systemic circulation suggests a new, previously unreported subset of leptomeningeal pores that require further investigation. The new mechanistic model of particle transport in the leptomeningeal space supports the initial idea of the study and suggests further development in accordance with the original plan.				
15. SUBJECT TERMS Neoplastic meningitis, leptomeningeal space, viral oncolysis, cerebrospinal fluid, drug delivery				
16. SECURITY CLASSIFICATION OF: a. REPORT U b. ABSTRACT U c. THIS PAGE U		17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 35	19a. NAME OF RESPONSIBLE PERSON USAMRMC
				19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION:

The goal of the proposed study is to develop novel, safe and efficient therapy for neoplastic meningitis. Neoplastic meningitis is a devastating complication of breast cancer caused by the spread of breast cancer cells into layers of tissues surrounding the brain that are normally filled with fluid. the space between two layers of isolating tissues enveloping the brain where neoplastic meningitis develops in isolated from the rest of the body and is not accessible to drug molecules circulating in blood. This study is aimed at the development of a drug of a novel type that would not escape from the space where the cancer cells spread causing neoplastic meningitis. We propose that an engineered virus hrR3, capable of killing cancer cells but harmless for normal tissues, will stay between the isolating tissue layers where the cancer cells spread, and kill them much more effectively than conventional drugs. The objectives of this exploratory study are to determine whether the hrR3 suspensions can stay in the area surrounding the brain for a period of time sufficient to infect the meningeal cancer cells and evaluate the safety and estimate the most effective schedule of hrR3 administration. Finally, we will determine the efficacy of hrR3 in an animal model of neoplastic meningitis.

BODY:

Task: Prepare and fractionate viral suspensions of hrR3. Obtain at least 4 samples with mean hydrodynamic sizes from 120 to 1000 nm.
The objective of this task was to obtain samples of viral suspensions with different particle sizes to determine the size-dependence of particle spread and retention in the leptomeningeal space after the intrathecal injection. It was found that suspensions of hrR3 obtained by the co-PI consist of particles with hydrodynamic size distribution from 50 to 900 nm. Centrifugation was found to be inefficient as a method of fractionation, probably due to a variable characteristic weight of the particles. Therefore, to obtain a set of radiolabeled particles emulating viral suspensions with narrow size distributions, we developed a method for producing ^{124}I -labeled particles with cores made of amorphous silica and surfaces modified as needed for the

study. Further animal studies (PET imaging, below) were carried out using these particles.

To obtain relatively hydrophobic particles, amino-modified monodisperse amorphous silica nanoparticles, 50 to 2000 nm in diameter, (Corpuscular, Inc., NY) were modified with p-hydroxyphenylpropionic acid with full modification of the aminogroups. The modification was carried out in dimethylsulfoxide (DMSO) using a 2x excess of Bolton-Hunter reagent in the presence of 0.1% 4-Dimethylaminopyridine (DMAP) overnight. The particles were isolated by centrifugation and washed with DMSO twice and with water 5 times (1 ml/mg each solvent).

Hydrophilic pegylated particles were obtained on the basis of the same aminated amorphous silica spheres. The latter were modified generally as above but with a smaller amount of Bolton-Hunter reagent to modify 5% of the aminogroups and washed with DMSO. Then, the particles were incubated with an excess NHS ester of PEG, 10 kDa, (1 mg/mg particle material) in the presence of 0.1% DMAP overnight. They were isolated and washed as described above.

Smaller molecular probes were prepared on the basis of dextran B-512. The latter was conjugated with tyrosine (2% of monomer units) for labeling with ¹²⁴I. The resultant macromolecules with hydrodynamic diameters of 3.7 and 7.1 nm were used in animal studies.

Task: Label the particles with narrow size distribution and investigate their activity in cell cultures and retention in CSF in rats, by PET.

Originally this task was to be carried out using fractionated virions. However, the amorphous silica model developed as described above provided a much higher monodispersity, which is important for estimation of the functional size of the meningeal pores. Thus, the task was carried out using monodisperse amorphous silica particles and model macromolecules. The particles were labeled with ¹²⁴I as described in the enclosed papers (see Appendix) and used in animal studies, along with radiolabeled macromolecules and protein molecules of known hydrodynamic diameters.

The studies were carried out, as planned, in rats (in parallel studies, similar experiments were carried out in cynomolgous monkeys).

The data (described in more detail in the papers enclosed in Appendix) suggests that particle and macromolecule transport in the leptomeningeal space differs very significantly from the transport of small molecules. Our data demonstrates, in particular, the following features of leptomeningeal transport that are essential for the proposed application:

(1) The key factor defining the patterns of the initial particle distribution between the cerebral and spinal CSF is the volume of the intrathecal bolus. Our measurements suggest that the “distance” from the lumbar injection point to cisterna magna is ca. 0.1 ml in rats and 1.5 ml in ca. 4 kg monkeys. Injection of larger volumes result in the immediate delivery of the solute to the cerebral subcompartment of the leptomeningeal space.

(2) The key physiological factors defining further transport of the particles in the leptomeningeal space (and further into perivascular spaces) are tissue pulsation and particle surface structure. Hydrophobic particles do not spread in CSF, presumably due to their nonspecific binding to the arachnoid. Hydrophilic particles move in the leptomeningeal space freely. Their transport is non-diffusional and not unidirectional, which suggest that the particles are transported through pulsation-assisted remixing of CSF in the leptomeningeal space and in the perivascular (Virchow-Robin) spaces. The rate of remixing enables solute translocation from the lumbar site to cisterna magna within about 5 hours in both rats and monkeys. This makes the kinetics of delivery in both animal models scalable to humans, where analogous translocation time for agents used in cisternography was reported. Virions behave in CSF as hydrophilic particles, which enables the scaling of their pharmacokinetics to humans. To further develop the rodent model, we are working with Charles River Laboratories to validate a newly (on our request) developed cranially oriented lumbar injection port. This model is expected to enable us to generate pharmacokinetic data for oncolytic viruses scalable to human lumbar administration.

(3) Our functional investigation of the meningeal pores that drain CSF outside the leptomeningeal space shows the following:

(a) the functional diameter of the large meningeal pores is close to that determined in the structural electron microscopy studies, i.e., ca. 1 micron. The data strongly suggests that CSF is not drained into the lymphatic systems in primates. In rodents, there is no lymphatic drainage from the spinal CSF pool, and possibly some drainage from the cranial pool (requires further investigation).

(b) In addition to the well described large meningeal pores, there is a smaller, previously uncharacterized subset of pores with functional diameters in the nanometer range. The pores are probably transcytosis-related and require further investigation.

(c) The meningeal pores are present not only in the sagittal sinus but also in the spinal leptomeningeal space. The size of the spinal subset has not yet been investigated.

Overall, the data suggests a new mechanistic model of particle transport in the leptomeningeal space, which strongly supports the feasibility of the initial idea of the study and suggests further development in accordance with the original plan.

Task: Investigate the possibility of de-aggregating the viral suspensions without loss of activity

The initial experiments have shown that the particles don't disaggregate under mild treatment with ultrasound. The study will continue with addition of mild detergents when viral suspensions become available in larger volumes.

Task: Initiate PET imaging and photoimaging studies of viral expression in meningeal cancer cells – see the co-PI's Annual report.

Task (originally planned for second year):

Pharmacokinetics/pharmacodynamics modeling. The novel physiology of the leptomeningeal transport (see the above and materials enclosed in Appendix) requires analyzing the mechanisms of each stage of the particle transport in CSF in order to demonstrate the qualitative relevance of the animal model to humans and the quantitative scalability. In order to obtain the data necessary for the modeling, we initiated the development of the intrathecally ported rat model (see above). If successful, this model, which enables low volume bolus injection and infusion, will provide a variety of quantitative data.

KEY RESEARCH ACCOMPLISHMENTS:

- A new mechanistic paradigm of particle transport in the leptomeningeal space developed
- A previously uncharacterized subset of meningeal pores (or processes functionally analogous to pores) is demonstrated
- A mechanistic basis for scalability of the pharmacokinetics from the rodent model to larger animals and humans is established

REPORTABLE OUTCOMES:

Full size research paper:

1. Papisov M, Belov V, Fischman A.J., Belova E, Titus J, Gagne M., Gillooly C. Delivery of proteins to CNS as seen and measured by Positron Emission Tomography. *Drug Delivery and Translational Research* 2012, 2:201-209

Proceedings papers:

2. M. Papisov, V. Belov, A.J. Fischman, J. Titus, M. Gagne, P. Calias, T. McCauley, M. Heartlein. Delivery of enzyme replacement therapeutics to CNS in rats and monkeys as seen and measured by PET. 39th Annual Meeting of the Controlled Release Society, Quebec, Canada, July 2012
3. Belova E, Vallance L, Belov V, Gagne M, Gillooly C, Papisov MI. PET-based approaches to studying the size-dependence of leptomeningeal drug clearance. 39th Annual Meeting of the Controlled Release Society, Quebec, Canada, July 2012.
4. Belov V, Papisov M. A PET based method for real time monitoring of drug concentration in the liquid phase of the leptomeningeal compartment. 39th Annual Meeting of the Controlled Release Society, Quebec, Canada, July 2012.

Abstracts:

5. Papisov M, Belov V, Fischman A, Titus J, Gillooly C, Gagne M. PET imaging of macromolecule and particle delivery to the brain through intrathecal administration in rodents and non-human primates. 2012 SNM Annual Meeting, Miami, Fl.
6. Belov V, Fischman A, Bonab A, Papisov M. Assessment of the prompt !- coincidences background from the I-124 activity inside and outside FOV. 2012 SNM Annual Meeting, Miami, Fl.

Awards:

7. Outstanding Pharmaceutical Paper Award. Controlled Release Society, 2012.

<http://www.controlledreleasesociety.org/about/Awards/Pages/OutstandingPharmaceuticalPaper.aspx>

Degrees obtained:

8. Lloyd Vallance, MLA (Biotechnology), Harvard Extension School, 2012.

Grant applications:

9. R21 grant application "Modeling of leptomeningeal transport of macromolecules and particles", NIH, 2012
10. R01 grant application "Leptomeningeal drug delivery route", NIH, 2012

CONCLUSION:

The research completed to date demonstrates feasibility of the original idea of this exploratory project. The new physiological data further suggests that complete coverage of the leptomeningeal space with intrathecally administered oncolytic viral suspensions is possible, optimally with a large volume bolus. (Safety of large-volume intrathecal injection has been established by others.)

The obtained data, as well as the progress achieved by the co-PI's research team (see Annual Report W81XWH-11-1-0388) suggest that the project should continue in accordance with the original plan. A new animal model (rat, catheterized in lumbar area) will be added to the protocol upon completion of the preliminary evaluation.

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1. Papisov M, Belov V, Fischman A.J., Belova E, Titus J, Gagne M., Gillooly C. Delivery of proteins to CNS as seen and measured by Positron Emission Tomography. *Drug Delivery and Translational Research* 2012, 2:201-209
2. M. Papisov, V. Belov, A.J. Fischman, J. Titus, M. Gagne, P. Calias, T. McCauley, M. Heartlein. Delivery of enzyme replacement therapeutics to CNS in rats and monkeys as seen and measured by PET. 39th Annual Meeting of the Controlled Release Society, Quebec, Canada, July 2012
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5. Papisov M, Belov V, Fischman A, Titus J, Gillooly C, Gagne M. PET imaging of macromolecule and particle delivery to the brain through intrathecal administration in rodents and non-human primates. 2012 SNM Annual Meeting, Miami, Fl.
6. Belov V, Fischman A, Bonab A, Papisov M. Assessment of the prompt I^-I^+ coincidences background from the $\text{I}-124$ activity inside and outside FOV. 2012 SNM Annual Meeting, Miami, Fl.

APPENDICES:

Published papers and abstracts:

1. Papisov M, Belov V, Fischman A.J., Belova E, Titus J, Gagne M., Gillooly C. Delivery of proteins to CNS as seen and measured by Positron Emission Tomography. *Drug Delivery and Translational Research* 2012, 2:201-209
2. M. Papisov, V. Belov, A.J. Fischman, J. Titus, M. Gagne, P. Calias, T. McCauley, M. Heartlein. Delivery of enzyme replacement therapeutics to CNS in rats and monkeys as seen and measured by PET. 39th Annual Meeting of the Controlled Release Society, Quebec, Canada, July 2012
3. Belova E, Vallance L, Belov V, Gagne M, Gillooly C, Papisov MI. PET-based approaches to studying the size-dependence of leptomeningeal drug clearance. 39th Annual Meeting of the Controlled Release Society, Quebec, Canada, July 2012.
4. Belov V, Papisov M. A PET based method for real time monitoring of drug concentration in the liquid phase of the leptomeningeal compartment. 39th Annual Meeting of the Controlled Release Society, Quebec, Canada, July 2012.
5. Papisov M, Belov V, Fischman A, Titus J, Gillooly C, Gagne M. PET imaging of macromolecule and particle delivery to the brain through intrathecal administration in rodents and non-human primates. 2012 SNM Annual Meeting, Miami, Fl.
6. Belov V, Fischman A, Bonab A, Papisov M. Assessment of the prompt $\text{I}^{\text{-}}$ coincidences background from the I-124 activity inside and outside FOV. 2012 SNM Annual Meeting, Miami, Fl.
7. Copies of published posters

SUPPORTING DATA

N/A

Delivery of proteins to CNS as seen and measured by positron emission tomography

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E. Belova · J. Titus · M. Gagne · C. Gillooly

Published online: 16 June 2012
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Abstract Presently, there are no effective treatments for several diseases involving the central nervous system (CNS). While several novel molecular approaches are being developed, many of them require delivery of macromolecular or supramolecular agents to the CNS tissues protected by the blood–brain and blood–arachnoid barriers. A variety of approaches that are being developed for overcoming or bypassing the barriers are based on complex transfer processes. The delivery of biopharmaceuticals and other macromolecules and particulates to the CNS, especially through the leptomeningeal (intrathecal) route, includes a variety of stages, such as leptomeningeal propagation, drainage to the systemic circulation, and penetration into the CNS. The investigation of complex pharmacokinetics that includes convective, as well as diffusional and active transfer processes, greatly benefit from real-time non-invasive *in vivo* monitoring of the drug transport. Pharmacological positron emission tomography (PET) imaging, which enables such monitoring, plays an increasingly significant role in drug delivery and biopharmacology. PET is a powerful tool for quantitative *in vivo* tracking of molecules labeled with positron-emitting radionuclides. The high sensitivity,

format, and accuracy of the data (similar to those of conventional tissue sampling biodistribution studies) make PET a readily adoptable pharmacological technique. In contrast to the conventional studies, PET also allows for longitudinal nonterminal same-animal studies. The latter may not only improve the data statistics, but also enable preclinical studies (especially in large and/or rare animals) not feasible under the conventional approach. This paper is intended to demonstrate the character of data that can be obtained by PET and to demonstrate how the main patterns of the leptomeningeal route pharmacokinetics can be investigated using this method. Examples of data processing are taken from our recent studies of five model proteins in rats and nonhuman primates.

Keywords PET Imaging · Pharmacokinetics · Biopharmaceuticals · Macromolecules · Brain · Central nervous system · Drug delivery · Iodine-124

Introduction

The leptomeningeal route to the central nervous system (CNS) starts from drug administration (injection or infusion) into the cerebrospinal fluid (CSF) at one of the clinically feasible locations. The latter generally include lumbar spinal region and brain ventricles. Intrathecal lumbar (ITL) administration can be carried out via either needle insertion through an intervertebral disk or a surgically implanted catheter equipped with a subcutaneous injection port or a pump. Intracerebroventricular (ICV) administration is carried out through a surgically implanted cannula connected to an injection port anchored to the skull.

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Upon mixing with CSF, the drug is then transported with the latter and can be either delivered to the target CNS tissues (brain, spinal cord, and nerve routes) or drained to the systemic circulation along with CSF in which it is dissolved.

The interface of CSF with CNS is not protected by any barriers. The layer of pia mater lining the brain surface is not continuous, and the continuity of the leptomeningeal space with the perivascular (Virchow–Robin) spaces penetrating deep into the parenchyma [1] is the major prerequisite for the potential efficacy of this delivery route. The leptomeningeal route includes the following major transport stages: (a) convectional transport in the CSF, (b) drug penetration into the brain parenchyma and spinal cord, (c) transport inside the CNS tissues, (d) drainage outside of the leptomeningeal compartment, and (e) uptake by cells lining the leptomeningeal space or otherwise residing therein. The fraction of the drug reaching the target region(s) in the CNS depends on the kinetics of all these processes.

The ITL drug delivery route was originally developed for small molecules, primarily anesthetics [2]. The behavior of macromolecules and nanoparticles in the leptomeningeal space has not been extensively studied. Thus, the leptomeningeal route to CNS, although promising due to the absence of a CSF–CNS barrier, consists of processes that depend on many insufficiently studied (for large molecules and nanoparticles) factors working at various levels and on different time frames, such as, remixing of CSF by the pulsatile movement of CNS tissues, drainage of CSF, and intraparenchymal transport.

Investigation of the complex combinations of transfer processes, as above, would benefit from methods enabling: (1) whole-body quantitative registration of all transfer processes on all time frames; (2) real-time data acquisition in the same animal; and (3) using any animal as its own control, which removes the individual variances from the kinetic data.

Positron emission tomography (PET), as a powerful tool for quantitative in vivo imaging of the transport of pharmaceuticals labeled with positron-emitting radionuclides, meets the above requirements. With the growing number of drugs and drug delivery systems that have nontrivial pharmacokinetics, PET imaging will play an increasingly significant role in preclinical (especially nonhuman primate) and, possibly, human studies.

The present studies were intended to investigate the pharmacokinetics of human recombinant enzymes after intrathecal (IT) administration in rats and nonhuman primates, to evaluate the relevance of rodent vs. primate models, and develop methodology for fully quantitative non-invasive pharmacokinetics studies by PET with ^{124}I .

Experimental methods

Imaging principles

Radionuclide-based imaging methods generally surpass all other in vivo imaging methods in sensitivity, and presently deliver enough resolution to delineate small organs in humans and experimental animals, including rodents. However, PET, which is based on a photon pair registration principle [3], has two technical advantages that make it superior to methods based on single photon acquisition.

In all radionuclide-based methods, the image is built on the basis of the experimentally acquired set of “lines” (lines of response or LORs in PET), along which each gamma photon has traveled before hitting a detector (a scintillating crystal).

In single-photon methods, i.e., planar imaging and gamma photon emission tomography (SPECT), the lines are defined via the use of collimators—metal (usually lead) blocks with channels, absorbing all photons except the ones traveling along the channels (Fig. 1). In planar imaging, a 2D image is formed essentially from the density of scintillations in the collimator crystal. In SPECT, the collimator/detector pair (“head”) rotates around the source of radiation, and a 3D image is reconstructed from the acquired set of lines.

In PET (Fig. 2), gamma photons are produced as a result of annihilation of the positron emitted by the radionuclide used as a label. Annihilation results in two gamma photons with peak energy of 511 keV, traveling in exactly opposite directions. Simultaneous detection of the pair by two detectors (the latter usually positioned as a set of rings) produces the LOR. The method does not require a collimator and, thus, is much more sensitive than single-photon modalities, where photon loss in the collimator generally exceeds 99 % (higher at higher resolutions). Photon scatter in the

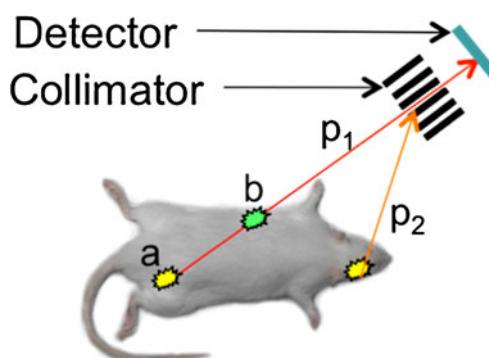


Fig. 1 Single-photon data acquisition. The detector registers only gamma photons (p_1) coming along the lines defined by the collimator, while other photons (p_2) are absorbed. It is impossible to determine whether photon p_1 came from point a , or point b , or any point in between. Thus, it is impossible to correct the data for photon absorption (attenuation) in the tissues

collimator is another factor complicating image formation in single-photon modalities.

The major advantage of PET, however, is in the relative simplicity of accounting for photon absorption in the tissues (attenuation). It is easy to see (Figs. 1 and 2) that there is no way of knowing from which point on the LOR a photon has come. Thus, in single-photon modalities, it is impossible to correct the data for absorption. In PET, the photons (as a pair) always pass the entire segment of LOR located between the detectors. Photon absorption in this segment can be easily measured experimentally, and thus the data can be readily corrected for attenuation. The attenuation data is produced from transmission images obtained using a rotating gamma radiation source or (in PET-computed tomography (CT)) from X-ray transmission data acquired by an X-ray CT imager.

There are several less significant differences in the data collection and processing, in particular those affecting resolution and artifact formation, but they are outside the scope of this paper. The combination of higher sensitivity and quantitative data acquisition are the two major advantages of PET. The quality of PET data (for a given tomograph), in turn, depends on many other factors, in particular on the positron emitting radionuclide used in the studies.

Iodine-124 as PET label

Imaging of slow PK, which is characteristic of many “large molecule” drugs and drug delivery systems, requires positron-emitting labels (radionuclides) with long physical half-lives. ^{18}F is by far the most widely used positron emitter due to the high diagnostic value of clinical PET imaging with $[^{18}\text{F}]\text{-fluorodeoxyglucose}$. However, the

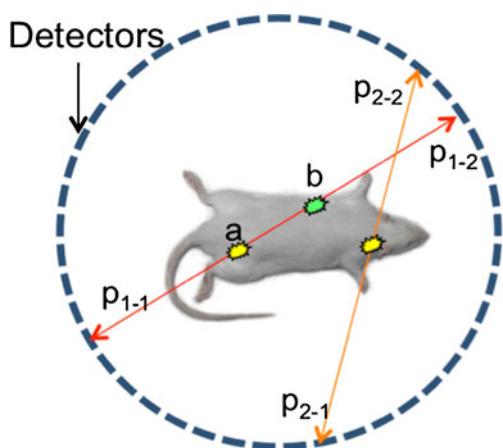


Fig. 2 Photon pair data acquisition in PET. Positron annihilation with emission of two gamma photons traveling in opposite directions along the same line. It is impossible to determine whether photon pair p_{1-1}/p_{1-1} came from point *a*, or point *b*, or any point in between. However, it is possible to correct the data for photon absorption in the tissues based on experimentally determined attenuation values

physical half-life of ^{18}F is only 110 min. To date, there are no satisfactory methods for labeling biomolecules (in aqueous phase) with this radionuclide. Thus, ^{18}F is a suboptimal choice for studies requiring long (>5–6 h) observations. Other radionuclides used in PET imaging, ^{11}C , ^{13}N , ^{15}O , ^{82}Rb and ^{68}Ga , have even shorter physical half-lives: 20, 10, 2, 68, and 1.3 min, respectively. Copper-64 has a somewhat longer physical half-life (12.7 h) and can be used for labeling of compounds premodified with a chelating group, but the insufficiently studied fate of the label *in vivo* after metabolism of the labeled compound presently makes it useful mostly for studying of the early stages of biomolecules pharmacokinetics.

Recently, ^{124}I , a cyclotron-produced radionuclide, has become commercially available. Among positron emitters available and suitable for PET, ^{124}I has the longest physical half-life of 4.2 days. This, combined with the well-investigated behavior of iodinated biomolecules and iodide *in vivo* [4–6], makes ^{124}I very attractive for long-term (several days) imaging studies.

The decay scheme of ^{124}I is complex; its emission spectrum includes high-energy positrons (23 %) and high-energy single photons (60.5 % at 603 keV). Both the high energy of the positrons and the presence of single photons in the range close to the 511 keV of annihilation photon pairs may lead to degradation of sensitivity, spatial resolution, and image quality. However, we have shown that proper use of ^{124}I provides fully quantitative data suitable for pharmacological research [7]. Therefore, ^{124}I (IBA Molecular, VA, USA) was the main radionuclide in our studies. The radionuclide was supplied in the form of sodium ^{124}I solution in 0.02 M NaOH, 0.3–2.7 $\mu\text{L}/\text{MBq}$. Nominal radiochemical purity: 95 % (<5 % of iodate and diiodate by high-performance liquid chromatography (HPLC)). Nominal radionuclidic purity is >99 % at calibration (<0.5 % of ^{123}I ; ^{125}I : none detected, by HPGe gamma spectroscopy). Chemical purity is Te <1 $\mu\text{g}/\text{mL}$ by UV–VIS spectroscopy.

Enzymes and labeling

Three human recombinant enzymes, idursulfase, arylsulfatase A, and sulfamidase, were produced and characterized by Shire HGT. The proteins were labeled with ^{124}I up to 185 MBq/mg and administered at various doses, via intravenous and intrathecal routes, in rats and (the former two proteins) in cynomolgus monkeys.

Protein labeling was carried out via direct iodination in the presence of Iodogen (Thermo Scientific Pierce, IL, USA). The iodination procedure was optimized for each protein to ensure >95 % radiochemical purity with preservation of enzyme structure, activity, and cell uptake characteristics. After the labeling, the proteins were briefly treated with metabisulfite (1 mg/ml, 1 min) to reduce iodoamines,

desalted on Sephadex G-25, and characterized by size exclusion HPLC with dual (gamma, UV) detection.

Imaging equipment

Imaging was carried out using either microPET P4 primate PET scanner (Concorde Microsystems/Siemens, TN, USA), or a custom PET/CT imaging system consisting of a MicroPET Focus 220 PET scanner and a CereTom NL 3000 CT scanner (Neurologica, MA, USA). In the latter system, the imagers were aligned and equipped with a custom imaging bed extending through both imagers along the alignment axis, ensuring reliable PET/CT image registration [8].

Both microPET P4 and Focus 220 worked in 3D mode and featured a 22 cm animal opening, axial field of view (FOV) 7.6 cm and transaxial FOV 19 cm. The scanner's detection systems enabled 2.5 mm (P4) and 2.1 mm (Focus 220) spatial resolution for ^{124}I . The energy window of the PET imagers was set for the entire study to 350–650 keV, and the coincidence timing window was set to 6 ns.

CereTom NL 3000 is a six-slice tomograph with high-contrast resolution of 0.4 mm (developed for human head imaging in ICU). The image acquisition settings were: tube voltage, 100 kV; tube current, 5 mA; resolution, 6 s/projection; and axial mode with slice thickness of 1.25 mm. Image pixel size was set to $0.49 \times 0.49 \times 1.25$ mm. The image sharpness was optimized to soft tissue. CT images were used for both anatomical reference and attenuation correction of the PET images. Fiducial markers (Eckert&Ziegler, Germany) were employed for PET/CT image co-registration that was carried out manually using ASIPROVM software (Siemens/CTI Concorde Microsystems, Knoxville, TN, USA).

PET data acquisition, histogramming, and reconstruction were executed with the aid of microPET software (Siemens Medical Solutions, Inc., Malvern, PA, USA). Corrections for isotope decay, detector dead time, random coincidences, and tissue attenuation were applied. CT image reconstruction was carried out using Neurologica software. All subsequent image processing and analysis were performed on nonhost workstations using the ASIPROVM software running under 32-bit Windows XP and Inveon Research Workplace 3.0 (Siemens Medical Solutions, Inc., Malvern, PA, USA) running under 64-bit Windows XP.

The PET data were reconstructed using a 3D ordered-subset expectation maximization/maximum a posteriori (OSEM3D/MAP) protocol with the smoothing resolution of 1.5 mm, nine OSEM3D subsets, two OSEM3D, and 15 MAP iterations. The data were also reconstructed with Fourier rebinning 2D filtered backprojection (FORE-2DFBP) [9] to ensure that the numerical data derived from OSEM3D/MAP and FORE-2DFBP reconstructed images were identical and thus to exclude the possible reconstruction artifacts

(none were identified). FORE-2DFBP was performed with a ramp filter cutoff at the Nyquist spatial sampling frequency (0.5 mm^{-1}). Whole body images were assembled of the acquired section images with a 12 mm overlap.

Animals

All animal studies were carried out in accordance with institutionally approved animal protocols.

Rats

IV administration Animals were set in a restrainer. A heparinized 3" catheter (BD 387334) was inserted into the tail vein and connected with a T connector (Abbott 1157218). Sodium pentobarbital, 35 mg/kg, was injected through the T connector cap and flushed with 0.5 mL saline. Then, animals were set on a MicroPET bed and injected with ^{124}I -labeled model protein through the T connector cap (flushed with 0.5 mL of saline) simultaneously with the start of dynamic imaging procedure.

IT administration Animals were anesthetized with sodium pentobarbital (50 mg/kg, IP injection). Nonsurgical intracisternal injection was carried out using a technique developed by Jeffers and Griffith [10]. Immediately after the injection, rats were placed on the MicroPET bed, and data were acquired for the cranial region for 20 min.

Nonhuman primates

Over the entire duration of the study, the animals were segregated from other NHPs and housed in a separate room at the MGH primate facility. At the housing site, the animals were sedated with Ketamine/Xylazine IM and then transported to the imaging site, where the animals were temporarily housed in standard NHP cages.

At the imaging site, each animal was first sedated with Ketamine IM (if and as needed), positioned on a custom polycarbonate imaging bed (MicroPET P4) or extended Focus 220 bed, and given continuous Isoflurane/O₂ anesthesia. Heart rate, breathing rate, and CO₂ content in the exhaled air were monitored continuously; isoflurane flow was adjusted as needed. Animals were given non-radioactive iodine (0.2 mL, 15 mM NaI) as a SC injection immediately before the study to suppress ^{124}I uptake in the thyroid. The radioiodinated proteins were administered intravenously (IV) or intrathecally.

IV administration A catheter equipped with a T connector was installed in the saphenous vein. The animals were set on

a MicroPET bed and positioned for dynamic imaging of the lower thoracic (heart and liver) area. A transmission image was acquired before the injection. Then, the protein solution was administered through the T connector cap and flushed with 1 ml saline simultaneously with the start of the dynamic imaging procedure.

ITL administration (direct injection) The animals were sedated; the injection point was shaved, wiped with 70 % alcohol, and treated with Betadine. Injections were carried out in a prone position with a support providing a vertically bent, exposed injection area (atlanto-occipital joint for ICM, L4-L5 area for IL). A 27 G 1" needle equipped with a 3" transparent catheter with a T-cap was inserted between the vertebra until CSF flow is detected in the catheter. A small volume (0.1–0.2 mL) of CSF was drawn through the catheter. Then, the ^{124}I -labeled model protein solution was injected through the T-cap, and the latter was flushed with 0.05 mL of the previously drawn CSF. The needle was withdrawn and the injection site was immediately imaged.

ITL and ICV administration (catheterized animals) The animals with surgically installed catheters equipped with subcutaneous injection ports (Northern Biomedical Research, MI, USA) were sedated; skin over the injection port wiped with 70 % alcohol, and treated with Betadine. Animals were set on the imaging bed in supine position. Injections were carried out through a 27 G 1" needle equipped with a 3" catheter with a T-cap. A small volume (0.1–0.2 mL) of CSF was drawn through the catheter. Then, the ^{124}I -labeled model protein solution was injected through the T-cap, and the latter was flushed with isotonic saline, 0.5 ml/kg body weight. The needle was withdrawn and the injection site was immediately imaged for 20 min.

Both rats and nonhuman primates were imaged following essentially the same procedure. First, data was acquired for 20 min for the body section including the injection site (or catheter opening for ITL and ICV injections), and a section including the heart and the anterior edge of the liver. (The data was subsequently used for dynamic reconstruction). Then, full body section-by-section PET scans were carried out, 5 min (rats, monkeys) or 10 min (monkeys) per body section. Scans were performed on various schedules, in most studies at 1, 2, 4, 8, 24, 48, 96, and 192 h in rats and 1–5 h continuously with subsequent (12 or 24), (24 or 48), and 72 h points in monkeys. The data was subsequently used for static reconstruction. The numerical data from manually selected regions of interest were processed to determine protein concentration in the tissues.

Other methods

Fluorescein isothiocyanate-labeled sulfamidase was utilized to investigate by photoimaging the microdistribution of the protein delivered to the brain in rats. The protein was injected into cisterna magna; animals were euthanized and the brains were cryosectioned 24 h after the injection. The cryosections were photoimaged (phase contrast, fluorescein 490/520 nm channel, custom blue 375/450 nm channel) without fixation or staining. Other control experiments were carried out in nonhuman primates (to be reported separately for each studied protein; partially published in [11]).

Results and discussion

Macromolecule transfer in CSF

The objective of our studies was to determine whether PET, as a quantitative imaging modality, could be used to investigate the general features of the leptomeningeal pharmacokinetics of macromolecules. Although the intrathecal route of drug delivery is not new, and vast data has been accumulated on the intrathecal administration of small molecules (mostly anesthetics), and several clinical applications have been successfully developed, both the properties of the prospective drugs and the mechanistic objectives of their delivery through the intrathecal route differ very significantly.

The main objective of the ITL administration of anesthetics is to achieve high drug concentration in the spinal cord and/or nerve roots locally to the injection point, whereas drug transfer to the cerebral leptomeningeal compartment can be detrimental to the subject (e.g., opiate drug transport to the respiratory center). In contrast, the target of enzyme replacement therapeutics and other macromolecular drugs is in the brain parenchyma, and their delivery to the cerebral leptomeningeal compartment is not undesirable but necessary.

The physicochemical properties of biomolecules generally should facilitate wide spread in the leptomeningeal compartment: they are too large to freely diffuse into the arachnoid and spinal cord parenchyma, and too hydrophilic to interact with the lipidic components of the tissue. Consequently, their behavior in CSF is expected to be entirely different from most anesthetics. On the other hand, the functions of the mesothelial and other cells have not been studied enough to predict how rapidly they will take up certain biomolecules dissolved in CSF. The flows and fluxes of CSF were also not sufficiently studied to predict how rapidly and in what directions biomolecules can be propagated in the leptomeningeal compartment from the injection site (ITL or ICV), although it is clear

that, considering the size of the molecules, diffusion cannot be expected to play a significant if any role in this process.

PET data demonstrated that in rats, all intracisternally administered proteins rapidly (within 5 min) spread over the entire cerebral CSF volume and into the proximal spinal leptomeningeal compartment (Fig. 3). In monkeys, the distribution process was dependent on the administered volume. Subcutaneous port administration generally suggests port flushing with relatively large volumes to ensure that the internal space of the port does not retain any significant fraction of the injected drug. The required volume for the ports used in this study (P.A.S. Port Elite, Smiths Medical ASD, Inc., MN, USA) to achieve $<1\%$ dose remaining in the port was found to be about 1 ml. This is probably because the flush volume mixes with, rather than displaces, the internal port volume. Since the studies were carried out in animals with a wide range of body weights, from approximately 2–5 kg, the flush volume was adjusted for body weight to achieve the same intraspinal flushing effect in large animals as in smaller ones and was equal to 0.5 ml per 1 kg body weight. This is well below the injection volume that, as literature data suggests, is safe for primates,

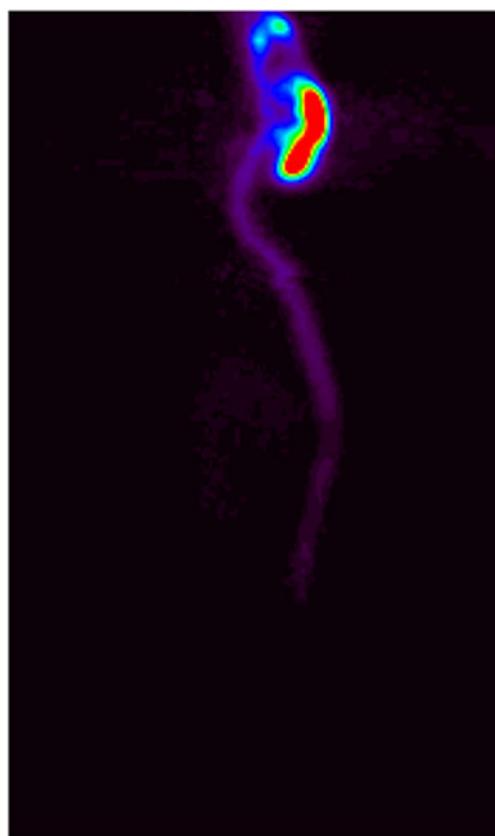


Fig. 3 Distribution of idursulfatase in rat 1 h after intracisternal administration. Dose, 10 mg/kg. Projection PET image (the sum of all sagittal slices). Relative color scale

both human and nonhuman (up to 30 % of the total estimated CSF volume [11]).

According to our data, lumbar protein administration with 0.5 ml/kg body weight port flush (monkeys), which is $<20\%$ of the estimated CSF volume, results in the immediate transfer of $55\pm20\%$ of the injected dose to the cerebral CSF. The movement of the protein in the spinal CSF is predominantly in the cranial direction, presumably because cranial and spinal leptomeningeal compartment (surrounded by bones) are not expandable, and the only expandable region of the dura is located in the cervical area, which accommodates the added volume.

Based on the imaging data, by 1–3 h, the administered protein fills all minor channels and tissue folds (Fig. 4). Lumbar administration in smaller volumes results in a slower but still efficient protein transfer to the cerebral CSF without deposition at the catheter opening site (Fig. 5, top). Visual analysis of the image shows that the protein spreads in both directions from the injection point, much faster than may be expected from a diffusion-based mechanism. We hypothesize that the transfer is convectional and is assisted by pulsatile movement of the arteries in the leptomeningeal space and CNS tissues.

Figure 5 (bottom) is a numerical graphic representation of the same data as is in the images. The graphs represent the amount of protein in the leptomeningeal compartment per unit of length of the spine from neck (left) to dorsal area. As can be clearly seen in the dorsal region, the rate of translocation of the “front” of the protein in the dorsal direction in this particular animal exceeds 10 mm per hour, which cannot be explained by diffusion, but is in agreement with the propagation of protein in CSF through constant remixing of CSF by local turbulences induced by the pulsation of tissues. In humans, the layer of CSF is thicker than in nonhuman primates, and the rate of propagation can be expected to be higher (which is important for the pharmacokinetics of ITL administered drugs and require further investigation).

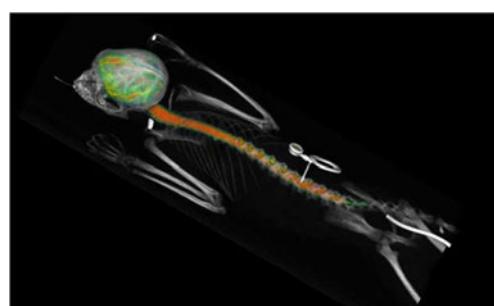


Fig. 4 Protein distribution 3 h after ITL administration through a subcutaneous port (center) connected through a subcutaneous catheter with the leptomeningeal space. PET/CT image. Color represents 3D map of the protein concentration as measured by PET

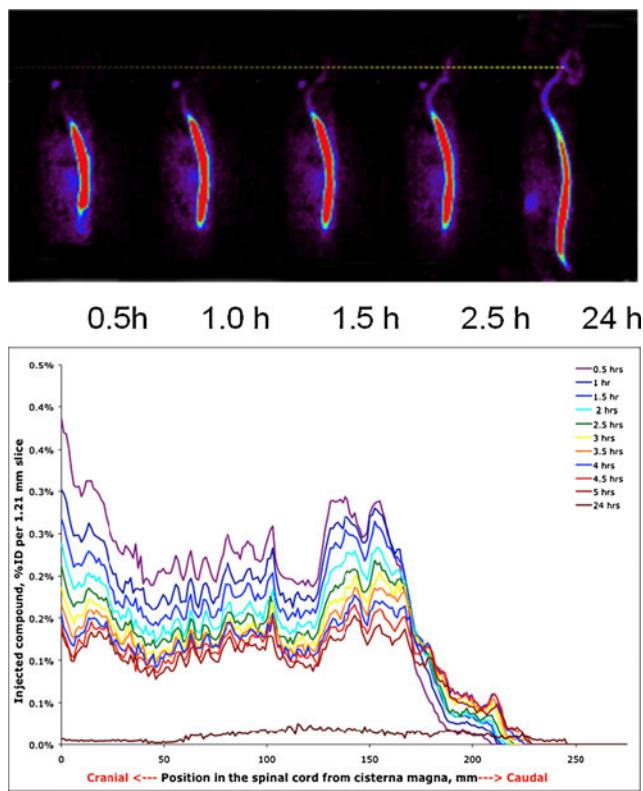


Fig. 5 *Top* visual representation of PET data on idursulfase (10 mg/animal) spread from the lumbar injection point in a monkey (color concentration). *Bottom* numerical graphic representation of the same data (% of ID per unit of spine length as a function of time)

The data in Fig. 5 suggests that the uptake of the administered idursulfatase by the leptomeningeal tissues is relatively low. However, this may not be predictive of the behavior of other biomolecules. Detailed data on the receptor-specific endocytosis in the leptomeningeal space would be of great value for the development of drugs intended for the leptomeningeal route.

Protein injected ICV rapidly (within 15 min) translocated into the cisternae and basal channels (Fig. 6). The resultant protein distribution in CSF was very similar to that of large volume ILT administration without the deposition in the distal spine (Fig. 7a, described in more detail in our earlier publication [12]). Thus, notwithstanding some previous data [13], there is apparently no significant CSF descent (contrary to the pulsatile remixing of CSF), at least in this species. This, however, may be a matter of individual variations and may be influenced by pathological conditions as well as the nature of the labeled solute used in the studies. The issue of CSF movement (especially the general directions of the fluid flux vs. solute movements) as a result of local turbulences generated by pulsation, appears to be insufficiently studied and requires further investigation. PET, as a quantitative imaging modality, can be instrumental in such studies.

Thus, both ICV and ITL administration routes appear to enable efficient delivery of macromolecules to the cerebral leptomeningeal space. The kinetics of the delivery depends on the injection (and port flush) volume.

Macromolecule penetration from CSF into the brain parenchyma

Rats The small size of the rat brain prevented data analysis beyond calculating whole brain averages. For the three model enzymes, the label content in the brain was 45, 70, and 35 % after ITL administration for Idursulfase, arylsulfatase A, and sulfamidase, respectively (for comparison, IV administration resulted in 0.20, 0.15, and 0.05 % of injected dose/g, respectively). Idursulfase was cleared from both the brain and spinal cord with a half-life of ~7 h, while for the other two enzymes the half-life was ~24 h. Photoimaging studies indicated enzyme deposition in pia mater as well as in the brain parenchyma (not shown).

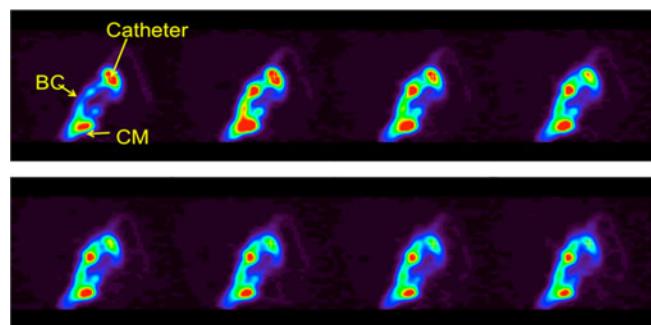
Monkeys In monkeys, protein penetration from CSF into the brain can be quantified from the imaging data in more detail due to the larger size of the brain. The rate of protein transfer was maximal during the first 2–5 h; by this time, a significant fraction (30–50 %, depending on the protein) was cleared from the CSF to the systemic circulation. Figure 7b illustrates the process of protein deposition in the brain for Idursulfatase.

Sequential PET imaging of the brain after the IT lumbar injection (Fig. 7b) demonstrated that I2S had moved from the CSF into the superficial (20–100 mcg/ml as estimated from PET data) and then into deeper brain tissues (3–20 mcg/ml). The inflow continued for 461 h, as exemplified by the differential (0.5–5 h) PET image in Fig. 7c. In the cranial segments, the clearance was faster (Fig. 7c), which is consistent with CSF drainage to the system predominantly in the arachnoid granulations of the superior longitudinal sinus [14]. No residual protein deposition was detected near the catheter opening.

Similar pharmacokinetics was observed for arylsulfatase and for nanoparticulate materials (not shown, will be published in detail elsewhere).

The mechanism(s) of protein translocation into the brain parenchyma from CSF require further investigation. The rate of translocation suggests participation of active transport mechanisms. To date, PET and photoimaging data suggest that two mechanisms may be at work, (a) pulsation-assisted translocation along perivascular (Virchow–Robin) spaces [15] and (b) axonal transport [16].

Fig. 6 Time course of ICV administration, 2 min per frame. PET dynamic reconstruction, sagittal slice through the catheter tip (*catheter*), central-basal channel (*BC*), and cisterna magna (*CM*)



Macromolecule clearance from CSF

Transfer of proteins dissolved in CSF to the systemic circulation started immediately after the injection, without a lag, which is not consistent with CSF drainage to the lymphatic system with subsequent transfer to the blood, and suggests transfer directly to the blood.

Lymphatic transfer would also suggest protein accumulation in lymph nodes draining cerebral, cervical and spinal regions. Any lymphatic accumulation would be readily detectable by PET. However, none of the studied monkeys ($n=20$) or rats ($n=64$) demonstrated any lymphatic accumulation along the spine, with exception of single nodes sentinel to the lumbar injection sites. In monkeys, no significant

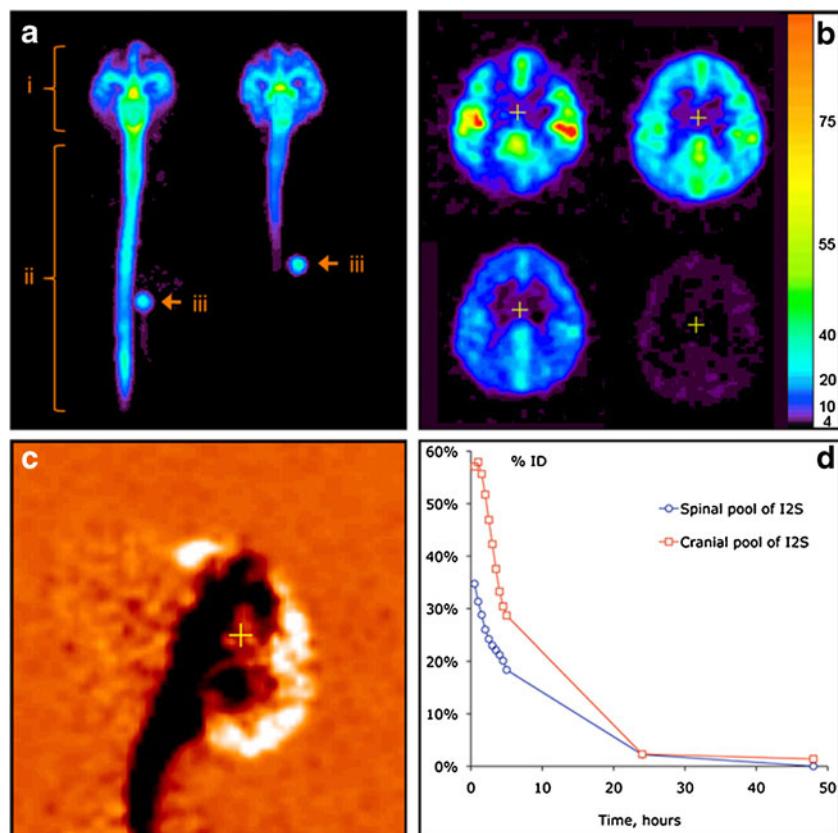


Fig. 7 In vivo distribution of ^{124}I -labeled I2S (3 mg/animal) in cynomolgus monkeys by PET. (a) Distribution of I2S administered through the lumbar (left) and ICV (right) catheters 30 minutes after the administration as demonstrated by a projection PET image (sum of all slices). Relative linear color scale. (b) The distribution of I2S in the brain at 0.5, 2.5, 5 and 24 hours after lumbar administration; PET image, 1.2 mm slice through the corpus callosum region in the plane parallel to the occipital bone. The color scale is calibrated in mg/ml of

I2S. (c) Changes in the cerebral I2S distribution between 0.5 and 5 hours after lumbar administration shown in monochrome linear color scale. The image was obtained by subtraction of the quantitative data matrix obtained at 5 hours from the one obtained at 0.5 hours. Neutral orange color represents no change. Clearance of I2S from the CSF is seen as black, and accumulation in the parenchyma and arachnoid as white color. (d) An example of single-animal dynamics of I2S clearance from the leptomeningeal compartment and CNS [12]

lymphatic accumulation was found in cervical lymph nodes as well. In rats, cervical and submandibular nodes showed some accumulation (generally <1 % of ID), but this could be a result of minor CSF leakages from the intracisternal injection sites and thus further investigation is necessary.

Thus, the PET data suggest that, at least in nonhuman primates, there is no physiologically significant CSF drainage into the lymphatic system, and in rats there is no such drainage from the spinal pool of CSF. This data further suggests that in primates, penetration of CSF through the cribriform plate into the olfactory epithelium is insignificant, because from there it would have to drain into the cervical lymph nodes, which has not been observed. There was no indication of significant radioactivity accumulating anywhere in or around the nasal airways at any time point as well.

Thus, the kinetics of protein drainage from the leptomeningeal space is in the agreement with direct drainage with CSF to the blood (presumably in arachnoid villi) [14], and the rate of protein transfer to the systemic circulation is in agreement with the rate of CSF exchange [17] (Fig. 7d).

Conclusions

PET provides a variety of image and quantitative data suitable for both visual and numerical analysis. Overall, our data demonstrate that the leptomeningeal (intrathecal) route is suitable and promising for protein delivery to the brain parenchyma, including both gray and white matter. Biologically significant levels of the studied proteins were found in all brain compartments. Considering the mechanistic context, this suggests that other macromolecules and nanoparticles may also be delivered to CNS via this route.

Several mechanistic aspects of leptomeningeal drug transport, such as parenchymal transfer, CSF drainage and mesothelial uptake, are important for understanding of the data, and warrants further investigation. The data demonstrate that the initial transfer of intrathecally administered proteins can significantly depend on the injection (and port flush) volume, which also warrants further investigation.

Acknowledgments This work was supported by NIH grant R21 CA152384, DoD grant BC100684, and grants from Shire HGT. Shire HGT also provided model proteins and imaging equipment.

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Delivery of enzyme replacement therapeutics to CNS in rats and monkeys as seen and measured by PET

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ABSTRACT SUMMARY

Hunter Syndrome, Metachromatic Leukodystrophy and Sanfilippo Syndrome are genetic lysosomal storage diseases (LSD) characterized by CNS degeneration as well as somatic symptoms. Presently, there are no effective treatments to alleviate the neurological component of these diseases. The objective of these studies was to investigate the pharmacokinetics of enzyme replacement therapeutics after intrathecal (IT) administration. The studies were also intended to evaluate the relevance of rodent models as compared with primate models and to develop methodology for fully quantitative non-invasive pharmacokinetics studies by Positron Emission Tomography (PET).

INTRODUCTION

Leptomeningeal transfer of intrathecally administered drugs to the CNS includes convectional transport in the cerebrospinal fluid (CSF) with subsequent penetration into the brain parenchyma and spinal cord. Concurrently, a fraction of the drug drains with CSF outside of leptomeningeal space (LMS) and yet another fraction may be accumulated in the leptomeningeal cells.

Thus, the intrathecal route to CNS relies on the processes depending on insufficiently studied factors, such as remixing of CSF by the pulsatile movement of CNS tissues, drainage of CSF (presumably to the blood through mesothelial pores and to the lymphatics via paraneurial interstitium), and intraparenchymal non-interstitial transfer. Investigation of complex combinations of transfer processes benefits from methods enabling: (i) whole-body quantitative registration of all transfer processes on all time frames, and (ii) real-time data acquisition in the same animal and using any animal as its own control, which removes the individual variances from the kinetic data.

PET, as a powerful tool for quantitative in-vivo imaging of the transport of pharmaceuticals labeled with positron-emitting radionuclides, meets the above requirements. With the growing number of drugs and drug delivery systems that have non-trivial pharmacokinetics PET imaging will play an increasingly significant role in preclinical (especially, non-human primate) and, possibly, human studies.

Imaging of slow PK that is characteristic for many “large molecule” drugs and drug delivery systems requires positron emitting labels (radionuclides) with long physical half-lives. Among positron emitters available and suitable for PET, ¹²⁴I has the longest physical half-life

of 4.2 days. This, combined with the well-investigated iodine behavior in vivo, makes ¹²⁴I attractive for long-term (several days) imaging studies. The decay scheme of ¹²⁴I is complex; its emission spectrum includes high energy positrons (23%) and high energy single photons (60.5% at 603 keV). Both the high energy of the positrons and the presence of single photons in the range close to the 511 keV of annihilation photon pairs may lead to degradation of sensitivity, spatial resolution and image quality. However, we have shown in our previous studies [1] that proper use of ¹²⁴I provides fully quantitative data suitable for pharmacological research.

The present studies were intended to investigate the pharmacokinetics of human recombinant enzymes after intrathecal (IT) administration in rats and non-human primates, to evaluate the relevance of rodent models vs. primate models, and develop methodology for fully quantitative non-invasive pharmacokinetics studies by Positron Emission Tomography (PET) with ¹²⁴I.

EXPERIMENTAL METHODS

Three human recombinant enzymes, idursulfase, arylsulfatase A, and sulfamidase, were labeled with ¹²⁴I and administered at various doses, via intravenous and intrathecal routes, in rats and (the former two proteins) in cynomolgus monkeys.

Imaging was carried out using either MicroPET P4 primate PET scanner (concorde Microsystems/Siemens) or a custom PET/CT imaging system consisting of MicroPET Focus 220 PET scanner and CereTom CT



Figure 1. Scheme of the non-human primate experiment (PET/CT image). Proteins were administered through a subcutaneous port (center) connected through a subcutaneous catheter with the leptomeningeal space. Color represents 3-dimensional map of the protein concentration as measured by PET (shown: 3 hours after the injection).

scanner (Neurologica, MA).

The general scheme of the non-human primate experiments is shown in Figure 1. Proteins were administered through a subcutaneous port connected through a subcutaneous catheter with the leptomeningeal space. Color represents protein concentration as measured by PET (shown: 3 hours after the injection).

In rats, intrathecal administration was carried out either via direct injection to cisterna magna through the Atlanto-Occipital joint, or through a surgically installed lumbar catheter.

Dynamic imaging data (0-20 min post injection) and multiple static images were acquired over up to 8 days after the injections. Acquisition, histogramming, and reconstruction were executed with the aid of Siemens software. CT images were used for attenuation correction and (as in Figure 1) for anatomical referencing. The numerical data from manually selected regions of interest were processed to determine the principal PK parameters.

FITC labeled sulfamidase was utilized to investigate by photoimaging the microdistribution of the protein delivered to the brain in rats [2].

RESULTS AND DISCUSSION

Overall, our data demonstrate that leptomeningeal (intrathecal) route is suitable for protein delivery to the brain parenchyma, including both grey and white matter. Biologically significant levels of the studied proteins were found in all brain compartments at doses found in other studies to be safe [3].

The data demonstrate that the initial transfer of intrathecally administered proteins significantly depends on the injection (and port flush) volume. The literature data suggests that administration of volumes up to 30% of the total estimated CSF volume is safe for primates, both human and non-human. According to our data, lumbar protein administration with 0.5 ml/kg body weight port flush (monkeys), which is <20% of the estimated CSF volume, results in the immediate transfer of ca. 50% of the injected dose to cerebral CSF. Lumbar administration in smaller volumes results in a slower protein transfer to the cerebral CSF (Figure 2). In both cases, the protein subsequently enters brain parenchyma. Protein penetration into the brain was quantified from the imaging data and the kinetics of it was found to be in agreement with CSF replacement rate. Thus, the rate of protein transfer was maximal during the first 2-5 hours; by this time a significant (30-50%, depending on the protein) fraction was cleared from CSF to the systemic circulation.

In rats, large volume (0.05 ml CSF withdrawal followed by 0.1 ml injection) IT administration to cisterna magna resulted in rapid protein distribution over the entire CSF volume, including distal spine. The initial label content in the brain was 0.20%, 0.15% and 0.05% of injected dose/g after IV and 45%, 70% and 35% after IT administration for Idursulfase, arylsulfatase A, and sulfamidase, respectively. Idursulfase was cleared from both the brain and spinal cord with a half-life of ~7h, while for the other two enzymes the half-life was ~24 h.

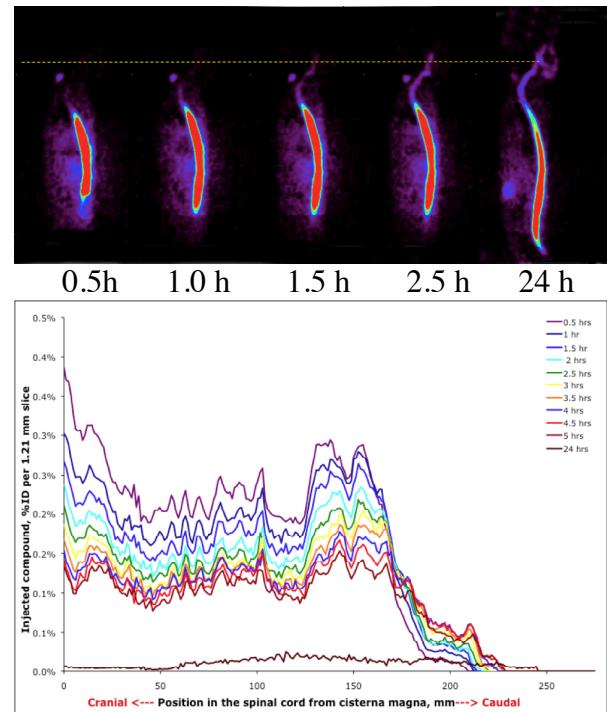


Figure 1. Top: visual representation of PET data on protein spread from the lumbar injection point in a monkey (color represents concentration). Bottom: numerical graphic representation of the same data (% of ID per unit of spine length as a function of time).

Photoimaging studies indicated enzyme deposition in pia mater as well as in the brain parenchyma.

PET also provided a variety of visual “4-D” data (“pharmacokinetics movies”) valuable for the initial analysis of the data, and a variety of data on protein transfer (e.g., along nerves and into lymphatics) that would not be accessible through other experimental methods.

CONCLUSIONS

The data suggest that leptomeningeal (intrathecal) route of administration can be beneficial for the treatment of CNS with biopharmaceuticals.

Several mechanistic aspects of leptomeningeal drug transport, such as parenchymal transfer, CSF drainage and mesothelial uptake, are important for understanding of the data, which warrants further investigation.

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ACKNOWLEDGEMENTS

This work was supported by NIH grant R21 CA152384, DoD grant BC100684, and grants from Shire HGT.

PET-based approaches to studying the size-dependence of leptomeningeal drug clearance

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ABSTRACT SUMMARY

Leptomeningeal space (LMS) provides a promising avenue for delivery of macro- and supramolecular drugs to central nervous system (CNS).¹ Leptomeningeal pharmacokinetics significantly depends on the turnover of the cerebrospinal fluid (CSF). Drug molecules dissolved in CSF escape to the systemic circulation along the CSF drainage routes. One of such routes is through leptomeningeal pores, which have not been sufficiently studied. The goal of this work was to identify approaches to predict the size dependence of the drug clearance from LMS.

We have investigated the process of clearance of macromolecules and particles of various sizes, from ca. 3 nm to 0.6 μ m, by Positron Emission Tomography (PET). The early data suggest that although the major population of leptomeningeal pores is large (>1 μ m), there is apparently another, previously unknown subset of smaller pores. The effective/functional size distributions of the pore populations as well as the anatomical location of the smaller subset are unknown and require further investigation.

INTRODUCTION

Successful therapy of many disorders involving CNS would benefit from effective delivery of macromolecular or supramolecular drugs to the brain. The intravenous route to CNS is not effective due to the barrier system physically and metabolically preventing the macromolecules from entering CNS tissues from the blood. The leptomeningeal (intrathecal) route provides an alternative approach through CSF, which contacts CNS tissues directly with no barriers between them. However, the fraction of the administered dose delivered to the CNS via this route, and, consequently, the efficacy of the treatment, will depend on the duration of the drug residence in CSF.

According to the current paradigm, CSF is produced by the choroid plexus in brain ventricles and drains into systemic circulation mainly in the arachnoid villi (AV) that are most abundant in the superior sagittal sinus. AV is a web-like structure of interlacing cords, continuing into the dura matter. In the sinus, the cords turn into delicate tissues covered by a layer of mesothelial cells, which separates CSF in the AV lumen from the blood in the sinus.² Based on scanning electron microscopy data, a pore-transfer mechanism of CSF passage via mesothelial cells was proposed³ where the pore size was estimated as being several micrometers.⁴

If CSF is drained through pores (whether static openings or active transcytosis-like processes) the rate of

macromolecule (and nanoparticle) clearance from CSF should depend on both the macromolecule and pore size distribution. The goal of this work was to investigate if PET imaging with radiolabeled macromolecules and particles administered to CSF provides an approach to pore size measurement. Here we report early data suggesting that quantitative PET imaging is an adequate method for investigating the functional size of the leptomeningeal pores.

EXPERIMENTAL METHODS

Clearance of macromolecules and nanoparticles from LAS of rats and non-human primates was investigated within broader studies of the pharmacokinetics of intrathecally administered proteins, linear polymers and supramolecular constructs (synthetic latexes, phage and virus particles).

PET with ¹²⁴I enables short- and long-term (several days) quantitative imaging studies of large molecule/particle pharmacokinetics.⁵ All proteins and preparations containing proteins were labeled via direct iodination with iodogen, generally as in our previous studies.^{1,5} Model dextrans were first modified with tyrosine (3% of monomer units) to introduce iodination sites. All radiolabeled preparations were treated with metabisulfite and desalted by either gel chromatography on Sephadex G-25 or (polystyrene particles) by centrifugation. Macromolecular preparations were studied by size exclusion HPLC with UV and gamma detection for radiochemical purity ($>95\%$).

The hydrodynamic diameters of the model preparations were determined by size exclusion HPLC using calibration by proteins with known hydrodynamic diameters: thyroglobulin (molecular weight $M_w=670$ kDa, Stokes radius $R_S=8.5$ nm), γ -globulin ($M_w=158$ kDa, $R_S=5.25$ nm), ovalbumin ($M_w=44$ kDa, $R_S=3.05$ nm), myoglobin ($M_w=17$ kDa, $R_S=1.9$ nm) and vitamin B₁₂ ($M_w=1.35$ kDa, $R_S=0.84$ nm).

All animal experiments were carried out in accordance with institutionally approved protocols. Normal 150–350 g Sprague-Dawley rats and 1–6 kg cynomolgus monkeys were used as animal models.

The labeled preparations were injected either directly into cisterna magna through the atlanto-occipital junction (rats) or through an intrathecal catheter equipped with a subcutaneous injection port. The amount of ¹²⁴I was ca. 0.1 mCi per rat and 0.5 mCi per monkey.

Animals were imaged on either MicroPET P4 (Concord Microsystems/Siemens) or a custom PET/CT system consisting of Siemens microPET Focus 220 and NeuroLogica NL3000 CereTom CT scanners. Dynamic

imaging was carried out for the first 20 minutes after the injection; then whole body scans were acquired at several time points.

The data were reconstructed into the image matrix with fixed slice thickness by iterative OSEM3D/MAP algorithm.

The label content in tissues was measured in manually drawn regions of interest. For each preparation, the data were tabulated; mean values and standard deviations were calculated where applicable.

RESULTS AND DISCUSSION

The early data obtained with proteins and virus and phage particles showed that a significant fraction (30-50%) of all studied preparations were able to penetrate from LMS to systemic circulation within 3-5 hours and accumulate in the same organs as after intravenous administration. The fact that large virions and phage particles (up to 0.6 μ m studied to date) were able to leave LMS suggested that there is a set of large channels acting as pores. However, significant differences in the clearance rates were observed also for much smaller 5-15 nm protein molecules, which suggested that there could be a smaller pore subset.

To exclude the influence of the possible specific macromolecule/particle interactions with cells, further experiments were carried out using fractionated tyrosin-modified dextrans and albumin-coated nanoparticles. After the injection, both polymers were rapidly and uniformly distributed over LMS (excluding lower spine). For the comparative analysis of polymer/particle clearance from CSF, total activity in cranial and spinal compartments at different time points was taken (example in Fig. 1).

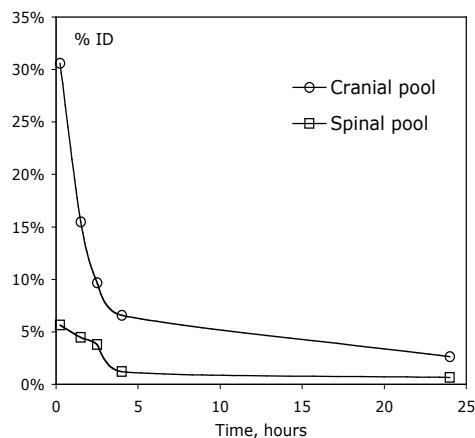


Figure 1. Dynamics of 3.8 nm polymer clearance from LMS, percentage of injected dose (ID) 15 min after injection.

For polymers, in particular, it was estimated that 15 min after injection, $31 \pm 6\%$ of 3.8 nm polymer and $42 \pm 10\%$ of 7 nm polymer were in the cranial pool. The dynamics of polymer clearance from CSF were noticeably different (Fig. 2). Within 4 hours after injection, almost 80% of the smaller polymer were transferred to the

systemic circulation, while only 50% of the larger 7 nm polymer were transferred at the same time point. After 24 hours, ca. 10% and 30%, respectively, still remained in LMS. Analogous studies with albumin-coated nanoparticles are currently in progress.

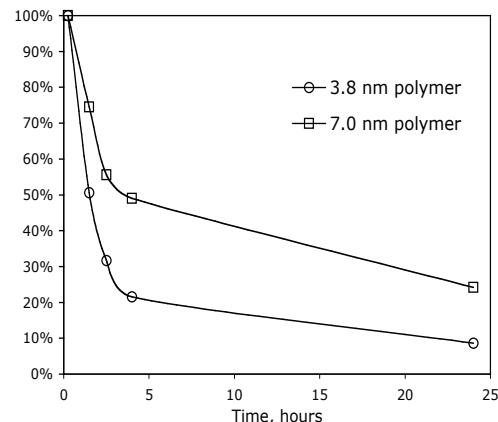


Figure 2. Dynamics of polymers clearance from cranial segment after intrathecal injection in percentage of initial (15 min after injection) uptake.

CONCLUSION

Clearance of macromolecules and particles from CSF into systemic circulation is size-dependent. The data obtained with particles suggests that there is clearly a set of transport channels functioning as very large pores; the size distribution needs further investigation. However, the size dependence observed for smaller, 3-15 nm molecules, suggests that there may be another subset of pores with smaller effective diameters, which has not been previously described.

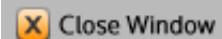
The obtained results are relevant to the development of drugs intended for delivery to CNS through leptomeningeal (intrathecal) route. The obtained preliminary data on the pore sizes warrant further investigation.

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ACKNOWLEDGMENTS

This work was supported by NIH grant R21 CA152384, DoD grant BC100684, and grants from Shire HGT, which also provided model proteins and equipment.

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Proof**CONTROL ID:** 1332707**PRESENTER:** Mikhail Papisov**CONTACT PERSON INFORMATION:** Mikhail Papisov, MGH Bartlett Hall 500R, 55 Fruit St, Boston, MA, United States, 02114-2696**Abstract Type:** Phy/Sci/Pharm**CURRENT TRACK:** Neurosciences**CURRENT CATEGORY:** Basic Science**Considered for:****AWARDS:****PRESENTATION TYPE:** Oral or Poster**Abstract****TITLE:** PET imaging of macromolecule and particle delivery to the brain through intrathecal administration in rodents and non-human primates**AUTHORS (LAST NAME, FIRST NAME):** Papisov, Mikhail¹; Belov, Vasily¹; Fischman, Alan J.³; Titus, James²; Gillooly, Caitlin²; Gagne, Matthew²**Institutional Author:****INSTITUTIONS (ALL):** 1. Massachusetts General Hospital & Harvard Medical School, Boston, MA, United States.

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ABSTRACT BODY:**Objectives :** Presently, there are no effective treatments for several diseases involving CNS. The objective of this study was to investigate the delivery of prospective therapeutics and model macromolecules and nanoparticles after intrathecal lumbar (IT) administration.**Methods :** Four recombinant human enzymes, model polymers of various molecular weights, virions and synthetic and phage particles, were labeled with 125I and administered IT to rats and cynomolgus monkeys. Dynamic imaging data (0-20 min post injection) and multiple static images were acquired over at least 48 hours after the injections. Images were analyzed to determine the rate and patterns of the label spread in CSF from the injection site and further into CNS.**Results :** After the injection, all molecules and particles were distributed in the CSF. Deposition at the injection site did not exceed deposition elsewhere in the leptomeningeal compartment. Penetration into both white and gray matter as well as major nerves were found by 5 hours after the injection. Penetration to the systemic circulation was detected early after the injection, the data suggests non-lymphatic size-dependent

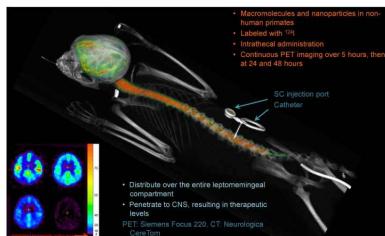
direct drainage to the blood with CSF.

Conclusions : The data suggest that IT administration of macromolecules and nanoparticles can be beneficial for the treatment of diseases involving CNS. The mechanisms and rates of macromolecule and particle clearance from CSF and penetration to the brain parenchyma require further investigation.

Research Support: NIH R21CA152384. DoD USAMRMC BC100684, and grants from Shire HGT.

(No Table Selected)

Supporting Data



References: Belov VV, Bonab AA, Fischman AJ, Heartlein M, Calias P, and Papisov MI. Iodine-124 as a Label for Pharmacological PET Imaging. *Molecular Pharmaceutics* 2011, v. 8 (3): 736–747

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Financial Disclosures

Financial Disclosure 2012:

Mikhail Papisov: No financial interest/arrangement

Vasily Belov: No financial interest/arrangement

Alan Fischman: No financial interest/arrangement

James Titus: No Answer.

Matthew Gagne: No Answer.

FDA Disclosure -Phy/Sci/Pharm (2): I do not have any applicable devices or drugs to disclose.

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A PET based method for real time monitoring of drug concentration in the liquid phase of the leptomeningeal compartment

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ABSTRACT SUMMARY

Positron Emission Tomography (PET) is a fully quantitative imaging modality able to accurately measure the concentration of a drug labeled with a positron emitting radionuclide in each part of the body in real time. Quantitative investigation of leptomeningeal (intrathecal) pharmacokinetics by PET is highly instrumental in the development of drugs with complex pharmacokinetics, such as ones intended for delivery to CNS via intrathecal route [1]. Limitations of PET relate to the areas where the drug resides in a liquid compartment and in fibers of tissue penetrating the liquid, as in the leptomeningeal space (LMS). This typically necessitates frequent sampling of the liquid to estimate the share of the solute in the total values measured by PET. The objective of this study was to develop an alternative method utilizing only PET imaging data.

INTRODUCTION

Drug transport from the intrathecal injection point to CNS tissues in cerebrospinal fluid (CSF) is complex and includes convective processes facilitated by the pulsatile motion of tissues and governed by the geometry of the liquid compartment and other factors. A fraction of the drug can be absorbed by the arachnoid tissues lining and penetrating the liquid compartment. In the PET image, these tissues are not resolved from the liquid compartment. Partial volume effect also complicates the situation, especially in preclinical studies in animals, where the layer of CSF, especially in the spine, is very thin. This necessitates periodic sampling of the liquid to measure drug concentration in it *ex vivo*. The total volume of CSF is not large, and frequent sampling with or without replacement can distort the data. If the sample volume is small, measurements are compromised due to the mixing of the sample with the residual liquid remaining in the port or/and collection catheter from the previous sampling or port flushing.

Considering the importance of resolving the liquid and solid phase components of the leptomeningeal space, we developed a PET based technique for selective investigation of CSF.

In this study, we propose measuring the concentration of the ¹²⁴I-labeled compound in CSF by PET in the catheter connecting the injection port and LMS.

In a separate study [2] we investigated the performance and quantification of PET imaging with ¹²⁴I and demonstrated its advantages for pharmacological research as well as limitations.

EXPERIMENTAL METHODS

Cynomolgus macaques with surgically implanted subcutaneous lumbar port (PAS Elite, Smith Medical, MN) were used in the study. The port was connected to the subcutaneous catheter inserted in the LMS. Port served for both drug administration and CSF collection. Model drugs labeled with ¹²⁴I were injected by slow bolus through the port, after which the port was flushed with isotonic saline.

Imaging was carried out in accordance with institutionally approved protocols using a custom imaging system consisting of a microPET Focus 220 scanner (Siemens) and CereTom NL 3000 CT scanner (NeuroLogica). The scanners were aligned to ensure reliable PET/CT image registration.

Focus 220 works in 3D mode and features a 22 cm animal opening, axial field of view (FOV) 7.6 cm and transaxial FOV 19 cm. The scanner's detection system provided a 2.1 mm spatial resolution for ¹²⁴I. CereTom NL 3000 is a 6-slice tomograph with high-contrast resolution of 0.4 mm (developed for human head imaging in ICU). Animals were sedated and set on a custom extended microPET bed in a supine position, head first, and positioned in the PET imager's field-of-view (FOV). For the duration of the imaging session the animals were given continuous Isoflurane/O₂ anesthesia.

The radiolabeled preparations were administered in 1 mL volume slowly over 1 minute, followed by flushing with saline, 0.5 ml/kg body weight. In most studies, the injected amount of ¹²⁴I was between 0.5 and 1 mCi (in the example shown below 386 µCi). The duration of PET data acquisition was 5 minutes per single bed position. A whole body CT scan followed the PET. The area of the injection port and catheter was scanned before and after collecting a CSF sample via the port. The list-mode data were reconstructed using OSEM3D/MAP iterative algorithm. The corrections for random coincidences, attenuation and scatter of annihilation photons, detectors dead-time, radioisotope decay as well as normalization to correct for the variation in detector efficiencies and distortion were applied to make the imaging fully quantitative. Attenuation and scatter corrections were based on CT images. Co-registration of the reconstructed PET and CT images was done manually using the tools implemented in the imager's software package. The images of the adjacent bed positions were stitched together using the software tool.

The essence of the method being developed is based on the fact that, although drug concentration inside the

catheter can not be measured due to the insufficient resolution, the total amount of radioactivity in a segment of the catheter can be. Thus, for each measurement only a relatively small volume of CSF can be withdrawn to the catheter (rather than through the catheter and port) for measurement.

Thus, a segment of the catheter of a certain length was selected in the reconstructed image as ROI. The total activity of ^{124}I in it was determined and divided by the volume of segment to obtain the concentration value. The internal volume of the catheter was measured experimentally. An isolated segment was filled with 10 μL of a contrast agent (Ultravist (Iopromide), 300 mgI/mL) with the aid of Hamilton syringe. The length occupied by liquid was measured in a CT image. The count-rate in the CSF sample withdrawn from the port was measured in a gamma counter (1480 Automatic Gamma Counter, Wallac Wizard 3"). To convert the count-rate into the activity concentration a cross-calibration curve between the gamma counter and the dose calibrator (AtomLab 100, Biodex Medical Systems) was used. PET scanner was calibrated for quantification against the same dose calibrator.

RESULTS AND DISCUSSION

A PET/CT image of injection port area is shown in Figure 1. CT image demonstrates the port and catheter configuration in the monkey. PET image shows the radiolabeled compound distribution in the CSF and its retention in the port. The total activity remaining in the port after flushing was 0.36 percent of the injected dose (ID). In other animal studies utilizing the same type of port, the maximal residual activity in the port was as high as 5.5% ID. After taking a CSF sample from the port, the total activity in the port increased to 1.91% ID.

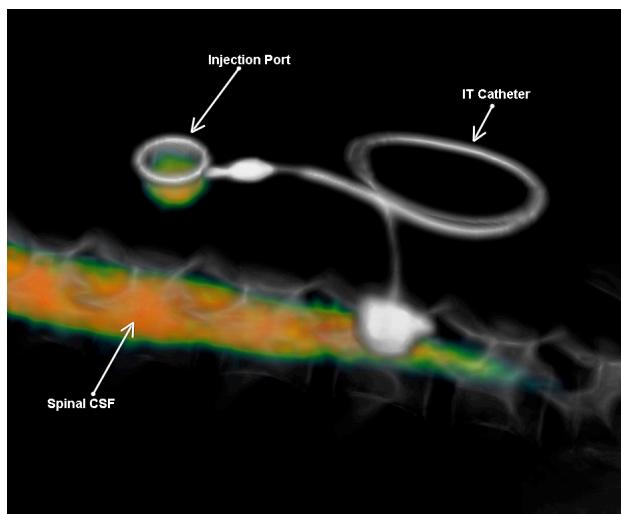


Figure 1. PET/CT image of the lumbar body region of the monkey 30 minutes after administration of the radiolabeled compound with the following flushing.

The catheter connecting the injection port with LMS has a double loop configuration with two straight regions,

near the port and near the entrance in the vertebrae. The connection of the catheter with a port has a variable thickness. Another straight region is very close to the spine, where high activity of the spinal CSF may distort the imaging data. The small portion of the distant curve of the catheter's loop (Figure 2) is most suitable for the measurements. A small region of it (5.572 mm) was taken as ROI in the PET image. The activity in the several analogous ROIs was averaged.

The diameter of catheter was found to be 0.512 mm. In this example, the ^{124}I concentration in the catheter measured by PET was found to be 46327 nCi/cc vs. sampled CSF that had 31265 nCi/cc as measured ex vivo.

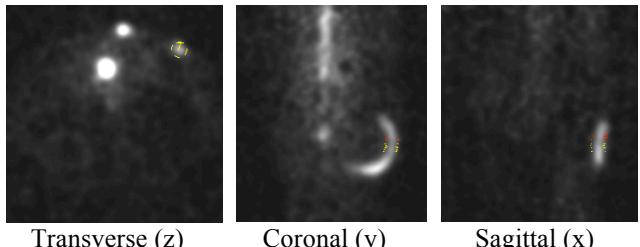


Figure 2. PET images (three projections) of the catheter filled with CSF after withdrawal of a CSF sample. 1.5 hr after injection of an ^{124}I labeled nanoparticles. ROIs used for activity calculation are shown by yellow and red lines.

The difference is between in-catheter and ex vivo measurements is expected and explained by the dilution of the CSF sample with the liquid remaining in the catheter and port after the previous flushing. Unless a very large volume of CSF is withdrawn from the port before sampling, the result of the measurement is distorted. CSF withdrawal to the catheter, on the other hand, provides undiluted fluid from LMS.

The proposed method requires certain corrections applied to the data during PET image acquisition, which should be taken into account in study planning.

CONCLUSION

PET imaging enables non-invasive quantification of the concentration of radiolabeled drugs in the liquid compartment of the leptomeningeal space.

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2. Belov VV, Bonab AA, Fischman AJ, et al. Iodine-124 as a label for pharmacological PET imaging. Molecular Pharmaceutics. 2011; 8(3): 736-747.

ACKNOWLEDGMENTS

This work was supported by NIH grant R21 CA152384, DoD grant BC100684, and grants from Shire HGT and Neurophage Pharmaceuticals.

 Close Window**Proof** Print**CONTROL ID:** 1332712**PRESENTER:** Vasily Belov**CONTACT PERSON INFORMATION:** Vasily Belov, 55 Fruit Street, White 427, Boston, MA, United States, 02114**Abstract Type: Phy/Sci/Pharm****CURRENT TRACK:** Instrumentation & Data Analysis**CURRENT CATEGORY:** Data Analysis & Management**Considered for:****AWARDS:** Center for Molecular Imaging Innovation & Translation (CMIIT) Young Investigator Award (YIA) Symposium**PRESENTATION TYPE:** Poster Only**Abstract****TITLE:** Assessment of the prompt γ -coincidences background from the I-124 activity inside and outside FOV**AUTHORS (LAST NAME, FIRST NAME):** Belov, Vasily¹; Fischman, Alan J.²; Bonab, Ali A.¹; Papisov, Mikhail¹**Institutional Author:****INSTITUTIONS (ALL):** 1. Massachusetts General Hospital, Harvard Medical School and Shriners Hospitals for Children, Boston, MA, United States.

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ABSTRACT BODY:

Objectives : Quantitative accuracy of I-124 is very important for pharmacological PET studies. Considering that nearly half of all positron decays of I-124 are accompanied with a 603 keV γ -photon in the same cascade, and detection of such photon with one of the annihilation photons may result in a false event registration, image quality and quantitative accuracy may be degraded if these events are not taken into account. Besides, additional photons causing γ -coincidences come from the label decaying in the organs positioned outside of the field of view (FOV). Hence, the background-generating effects of both the inside and outside FOV activities (FOVA and OFA, respectively) should be investigated.

Methods : Imaging was carried out using MicroPET Focus 220 scanner (Siemens). A volume phantom filled with 251 μ Ci (in the FOV) and a point source containing 150 μ Ci (outside FOV) of I-124 were used in the studies. The background in the reconstructed image and the background average number of counts per voxel in the SSRB-sorted sinogram were measured by drawing the ROIs within the 2 cm edge of the FOV. The prompt γ -photon fraction was then calculated. The data were analyzed in two energy ranges: 350-650 keV and 350-600 keV.

Results : The FOVA resulted in a uniform unstructured background. The average activity concentration in the reconstructed image was 15.08 nCi/cc per 100 μ Ci of the FOVA. The prompt γ -photon fraction was 0.23. The OFA at 5 mm distance from the edge of the FOV leaded to the highest non-uniform background, with the

maximum value being 16.98 nCi/cc per 100 μ Ci of the OFA. The effect of OFA at larger distances degraded in accordance with the mathematical model. Reduction of the upper energy threshold resulted in a ca. 15% lower background.

Conclusions : Prompt γ -coincidences are a significant feature of the non-pure positron emitters and should be taken into account in image reconstruction and analysis.

Research Support: NIH, DOE

(No Table Selected)

Supporting Data

(No Image Selected)

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Financial Disclosures

Financial Disclosure 2012:

Vasily Belov: No financial interest/arrangement

Alan Fischman: No financial interest/arrangement

Ali Bonab: No financial interest/arrangement

Mikhail Papisov: No financial interest/arrangement

FDA Disclosure -Phy/Sci/Pharm (2): I do not have any applicable devices or drugs to disclose.

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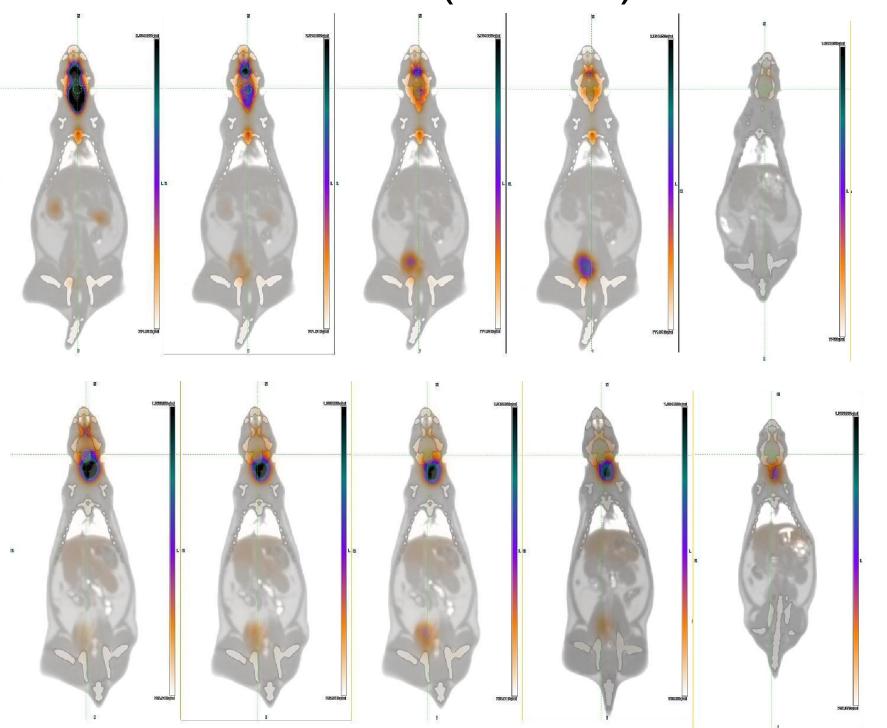
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PET/CT images of Rats injected with radiolabeled polymers and nanoparticles

3.7 nm dextran (top)

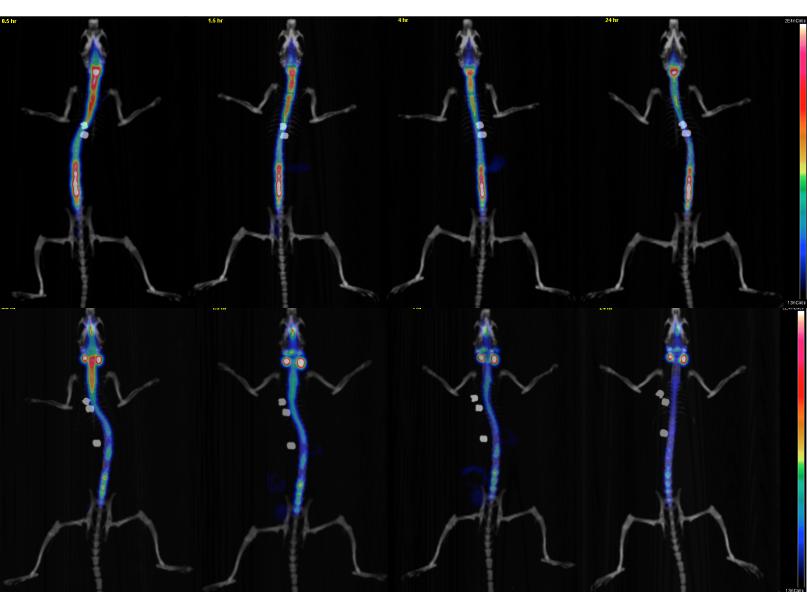
7.1 nm dextran (bottom)



$T=0.25 \text{ hr}, 1.5 \text{ hr}, 2.5 \text{ hr}, 4 \text{ hr}, 24 \text{ hr}$

187 nm particle (top)

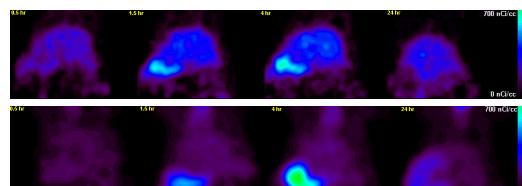
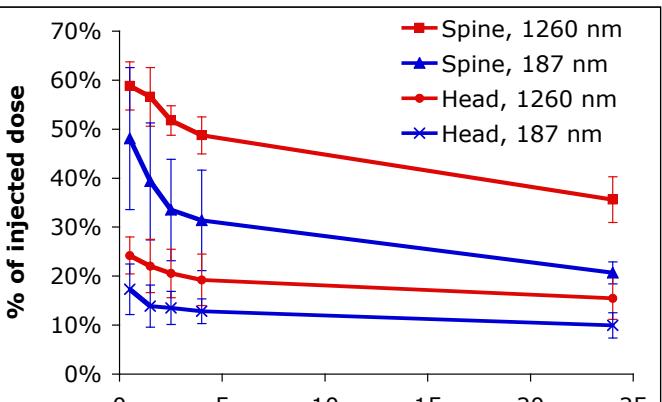
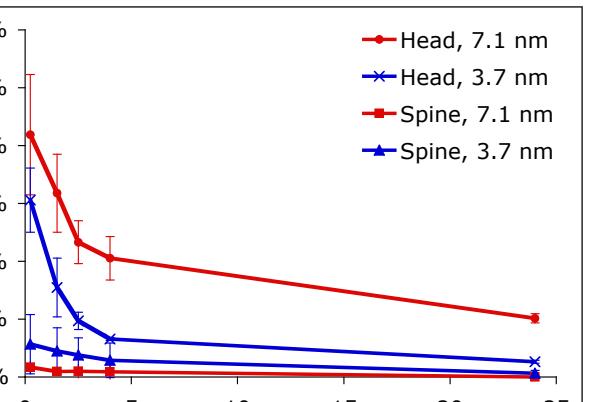
1260 nm particle (bottom)



$T=0.5 \text{ hr}, 1.5 \text{ hr}, 2.5 \text{ hr}, 4 \text{ hr}, 24 \text{ hr}$

Coronal view (PET) of a Rat liver after injection of particles of 187 nm (top) and 1260 nm (bottom)

The fraction of the administered dose in the cerebral (Head) and spinal (Spine) compartments



$T=0.5 \text{ hr}, 1.5 \text{ hr}, 2.5 \text{ hr}, 4 \text{ hr}, 24 \text{ hr}$

Kinetics in the





A PET-Based Method for Real-Time Monitoring of Drug Concentration in the Liquid Phase of the Leptomeningeal Compartment

Vasily Belov, Mikhail Papisov

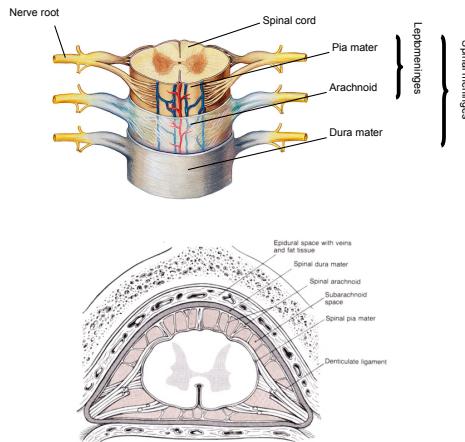
Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114-2696



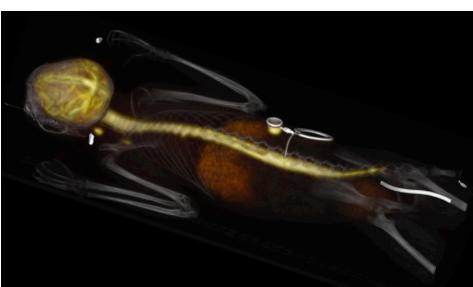
Introduction

- Cerebrospinal Fluid (CSF) surrounds the brain and spinal cord circulating in the leptomeningeal space (LMS). The absence of barrier between the CSF and brain/spinal cord parenchyma makes intrathecal space a very promising route for delivery of drugs to the CNS.
- Various arachnoid tissues lining the LMS and protruding into the CSF can absorb some fraction of the drug. The functions of cells in these tissues are poorly studied so the rate of uptake by them cannot be predicted or estimated for a certain biomolecule dissolved in CSF. Meanwhile estimating the drug fractioning between the liquid and solid components of the LMS is very important for pharmacokinetic analysis of drug distribution.
- Quantitative investigation of leptomeningeal pharmacokinetics by positron emission tomography (PET) is highly instrumental. However the resolution of PET is not sufficient to resolve components of LMS. This necessitates periodic sampling of CSF via the injection port to measure drug concentration in the fluid ex vivo. Such measurement is compromised due to the mixing of the sample with the residual liquid remaining in the injection port after the previous sampling or port flushing. We propose the alternative, more accurate method for measuring drug concentration in the collection catheter by PET.

Anatomy of the Leptomeningeal Compartment



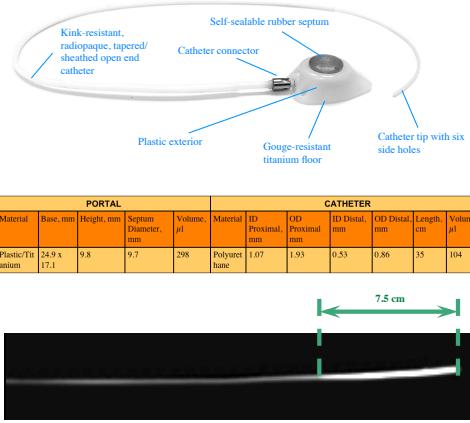
A PET/CT Imaging of Intrathecal Drug Delivery



PET/CT image. Monkey, 3.5 hours post IL injection of 1 mCi

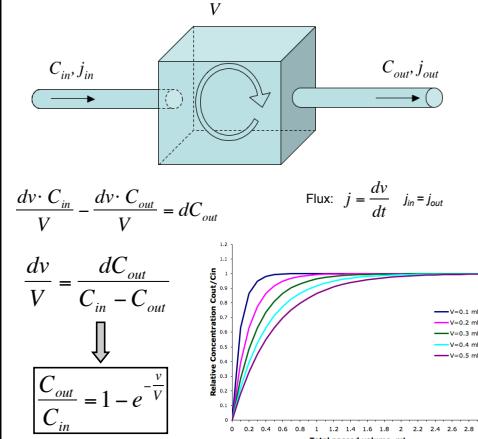
- The port is used for both drug administration and CSF sampling

The Port and Catheter Configuration

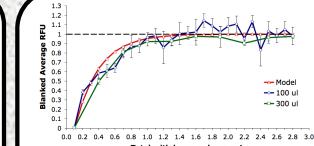


Results

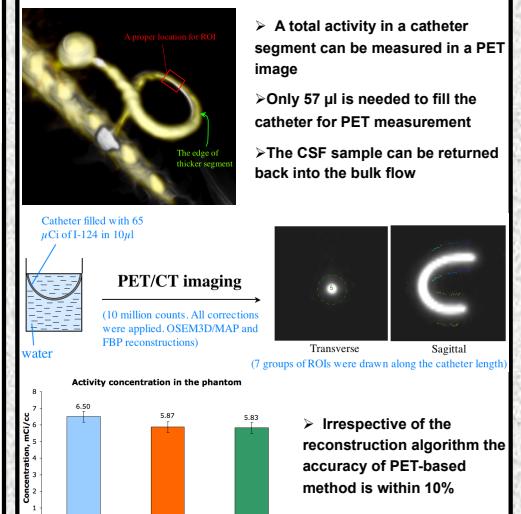
Modeling the Port Sampling



Ex Vivo Study of Port Sampling

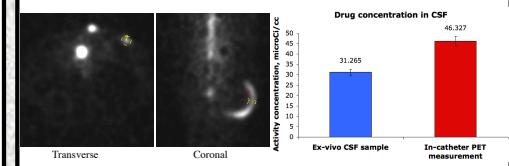


A PET-Based Method and Its Accuracy



- Irrespective of the reconstruction algorithm the accuracy of PET-based method is within 10%

Animal Study



- CSF sampling through the port is compromised due to the sample dilution by the residual liquid remaining in the port

Conclusions

- We showed that sample collection via the injection portal is inaccurate unless a very large volume is taken.
- PET imaging enables non-invasive real-time quantification of the concentration of radiolabeled drug in the liquid phase of LMS.

Acknowledgments

This work was supported in part by NeuroPhage Pharmaceuticals (Cambridge, MA), which also provided a model drug